IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: CANTOR *et al.*

Serial No.:   09/030,571

Confirmation No.:   7542

Customer No.:   20985

Filed: February 24, 1998

For: *POSITIONAL SEQUENCING BY HYBRIDIZATION*

Art Unit: 1634

Examiner: Forman, B. J.

DECLARATION

Mail Stop **RCE**
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, FRANK J. MISKIEL, declare as follows:

1)   I am an associate working with Stephanie Seidman, who is applicant's attorney responsible for prosecuting the above-captioned application.

2)   The amendment to the specification, which inserts material after the paragraph on page 12, line 12, describing the degenerate probes of Macevicz with more particularity, consists of the same material on page 3, line 37 through page 6, line 9 of International Patent Application PCT/US89/04741, incorporated by reference in its entirety in the referencing application (see page 12, lines 2-3), except for the following changes required for grammatical continuity with the instant application. The changes to the disclosure of International Patent Application PCT/US89/04741, published as WO 90/04652, are provided below.

At page 3, line 37, the recitation "If" is replaced by the recitation
        —Macevicz teaches degenerated probes where, if—

At page 4, line 15, the recitation "Formula II" is replaced with —Formula II
        below—

At page 4, line 18, the recitation "probe, Martin" is replaced with —probe
        (Martin—

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At page 4, line 19, the recitation "(1985)." is replaced with –(1985)).–

At page 5, line 17, the recitation "of the invention" is deleted
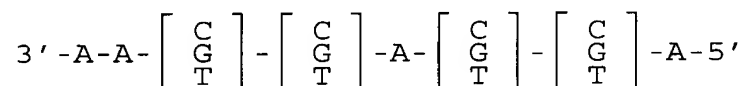
At page 5, lines 21-22, the recitation "an important feature of the invention is that" is deleted

At page 5, line 28, the recitation "of the invention" is deleted.

These changes are also shown below to provide context for the changes.

Insertions are underlined, **deletions are in** ~~strikethru~~ **or [[bracketed]]:**

Macevicz teaches degenerated probes that, if [[If]] the probes were each 8 bases long, a member probe of the adenosine subset can be represented as follows:

$$3'-A-A-\begin{bmatrix}C\\G\\T\end{bmatrix}-\begin{bmatrix}C\\G\\T\end{bmatrix}-A-\begin{bmatrix}C\\G\\T\end{bmatrix}-\begin{bmatrix}C\\G\\T\end{bmatrix}-A-5'$$

Formula I

The symbol $\begin{bmatrix}C\\G\\T\end{bmatrix}$ means that any of the bases C, G, or T may occupy the position where the symbol is located. Thus, the above probe has a multiplicity, or degeneracy, of $1 \times 1 \times 3 \times 3 \times 1 \times 3 \times 3 \times 1$ or 81. When it is clear from the context which subset is being considered, the above notation will be simplified to AAOOAOOA, where A represents deoxyadenosine and O represents the absence of deoxyadenosine.

Preferably, base analogs are employed in the oligonucleotide probes whose base pairing characteristics permit one to reduce the multiplicity of the probe. For example, in the probe of Formula II below, because deoxyinosine (I) forms nearly equally strong base pairs with A and C, but forms only a weak or destabilizing base pair with either G or T, deoxyinosine can replace G and T in the probe [[,]] (Martin *et al.*, Nucleic Acids Research, Vol. 13, pgs. 8927-8938 (1985)). Thus, a probe equivalent to that of Formula I, but which has a much lower multiplicity (*i.e.* only 16) can be represented as follows:

$$3'-A-A-\begin{bmatrix}I\\C\end{bmatrix}-\begin{bmatrix}I\\C\end{bmatrix}-A-\ 5'$$
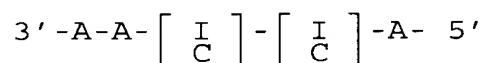
Formula II

Generally, base analogs are preferred which form strong base pairs (*i.e.*, comparable in binding energy to the natural base pairs) with two or three of the four natural bases, and a weak or destabilizing base pair with the complement of a fixed base (defined below). Such base analogs are referred to herein as degeneracy-reducing analogs.

It is not critical that the probes all have the same length, although it is important that they have known lengths and that their sequences be predetermined. Generally, the probes will be fixed at a predetermined number of positions with known bases (not necessarily of the same kind), *e.g.* as the A in Formula I, and the remaining positions will each be filled by a base randomly selected from a predetermined set, *e.g.* T, G, and C as in Formula I, or I and C as in Formula II. The positions in a probe which are nondegenerate in their base pairing, *i.e.* have only a single natural base, are referred to herein as fixed positions. The bases occupying fixed positions are referred to herein as fixed bases. For example, the fixed bases in the probes of Formulas I and II are deoxyadenosine at positions one, two, five, and eight with respect to the 3' end of the probe.

Generally, sets and/or subsets ~~of the invention~~ each contain at least one probe having a sequence of fixed and non-fixed positions equivalent to that of each permutation of a plurality of fixed and non-fixed positions less than or equal to the length of the probe. That is, ~~an important feature of the invention is that~~ the probes collectively contain subsequences of fixed and non-fixed positions (up to the total length of the probe), which correspond to every possible permutation of fixed and non-fixed positions of each of a plurality of combinations of fixed and non-fixed positions, the plurality including combinations containing from zero to all fixed positions. For example, consider a subset of probes ~~of the invention~~ that consists of 8-mer probes whose fixed positions contain only deoxyadenosine and whose initial (*i.e.*, 3'-most) position is fixed. The probes of Formulas I and II are members of such a subset. Within such a subset, there is at least one probe having a subsequence of fixed and non-fixed positions in positions 2 through 8
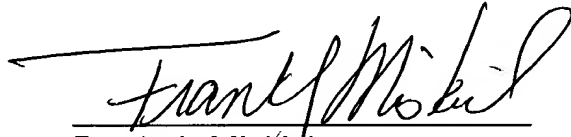
which corresponds to each possible permutation of fixed and non-fixed positions for subsequences having no fixed positions (one such permutation: A0000000), one fixed position (seven such permutations, *e.g.* A000A000), two fixed positions (twenty-one such permutations, *e.g.* A00AA000), three fixed positions (thirty-five such permutations, *e.g.* A0000AAA), four fixed positions (thirty-five such permutations, *e.g.* A0AAAA00), five fixed positions (twenty-one such permutations, *e.g.* AAA00AAA), six fixed positions (seven such permutations, *e.g.* AAAA0AAA), and seven fixed positions (one such permutation: AAAAAAAA). Thus, the subset has at least $1 + 7 + 21 + 35 + 35 + 21 + 7 + 1 = 128$ members.

No ne matter is added.

<div align="center">* * *</div>

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

17 August 04
Date

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